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STUDIES ON THE DISINFECTANT PROPERTIES OF G-11

(HEXACHLOROPHENE)

BY

SAKARI EINOLA

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MERCATORIN KIRJAPAINO
HELSINKI, FINLAND

FROM THE DEPARTMENT OF MEDICAL MICROBIOLOGY,
UNIVERSITY OF TURKU

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PREFACE

The subject of the present investigation was suggested to me by Professor Eero Mustakallio, M.D., Chief of the Department of Medical Microbiology, University of Turku. He has closely followed the progress of my work and his benevolence and continued interest have been invaluable. For this and for the opportunity of working in his department, I wish to record my deep gratitude to him.

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For the solubility determinations, I am indebted to Mr. K. Passinen, B.Sc. in Chem. Engineering.

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Turku, May 1955. *Sakari Einola*

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INTRODUCTION

In a search for antiseptic compounds that could be incorporated in soap without losing their bactericidal properties, Gump (1939) synthesized a number of halogenated diphenylmethanes, of which one designated as G-11 was later found to fulfill best those requirements for such a compound which Gump (1945) stated as follows: It should retain its germ-killing powers in soap. It should not react with the soap constituents, or with the moisture contained in soap. It also should not be influenced by the free alkali of the soap nor the alkalinity produced by the hydrolysis of the soap in the water. It should not be volatile and should not have a disagreeable odour. It should be relatively nontoxic and should not be irritating to the skin or be a sensitizer. It should not stain the skin or cause spots on the laundry.

G-11 or hexachlorophene, the generic term accepted for it by the Council of Pharmacy and Chemistry of the American Medical Association (1948), has in the light of numerous investigations proved to be an effective skin germicide in admixture with soap and synthetic detergents. During the past decade its use in soaps and other detergents has expanded greatly and it has been found to fulfill in practical applications all those requirements set by the inventor.

According to current theories, surface active compounds which are composed of long hydrophobic chains bearing hydrophilic end groups form aggregates called micelles when their concentrations in aqueous solutions exceed certain limits. The concentration at which micelle formation begins is known as the critical micelle concentration of the surface active compound. Examples of such compounds, which are also termed association colloids, are the alkali salts of long-chain fatty acids, e.g. potassium laurate ($CH_3(CH_2)_{10}COOK$), and of monoalkyl sulphates, e.g.

sodium lauryl sulphate ($CH_3(CH_2)_{11}OSO_2ONa$), and Tween 80 (polyoxyalkylene deriv. of sorbitan monooleate). The critical micelle concentration varies with the length and the type of the long hydrophobic chain and with the nature of the hydrophilic group. The critical micelle concentration increases when the temperature is raised, while addition of electrolytes to the solution lowers the critical concentration. The formation of micelles in the solutions greatly influences the properties of these solutions. Thus, for instance, the surface tension of such a solution diminishes rapidly when the colloid content of the solution increases up to the critical concentration, but then undergoes a slow increase when more colloid is added.

Aqueous solutions of association colloids in which micelles are present are able to dissolve substantial amounts of many substances that are insoluble or sparingly soluble in pure water. In certain cases it has been observed that the solubility of such sparingly soluble compounds is somewhat greater also in colloid solutions in which the critical concentration has not been exceeded than in water; this has been attributed to the presence of elementary structures in the solutions. The solubility curve for the weakly soluble compound frequently undergoes an abrupt increase in slope in the vicinity of the critical concentration of the colloid. The solubilizing power of the colloid solution has been observed to increase with the length of the hydrophobic part of the colloid molecule. The solubilization phenomena are connected with the peculiar structure of the micelles which are composed of an inner core formed by the hydrophobic groups of several colloid molecules and surrounded by the hydrophilic groups which are oriented outwards against the water.[1]

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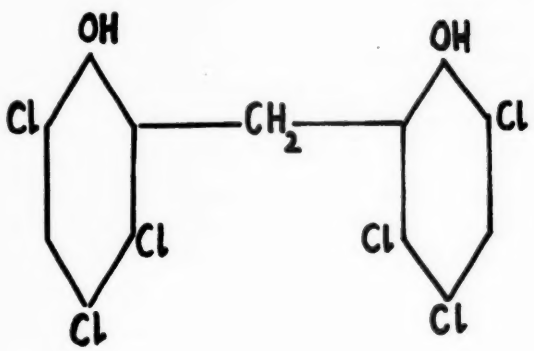
[1] For a more comprehensive review, see, e.g., the publications by Glassman (1948), Berry and Bean (1950) and Ekwall (1951), and the papers cited there.

LITERATURE SURVEY

G-11 (HEXACHLOROPHENE)

PHYSICO-CHEMICAL PROPERTIES OF G-11

G-11, the chemical name of which is bis-(3,5,6,-trichloro-2-hydroxyphenyl)methane, is a white, odourless, crystalline powder melting at 164—165°C. Its solubility in various solvents is shown in Table 1. The compound, whose structural formula is shown below, possesses weak acid properties by virtue of its phenolic hydroxyl groups.



TOXICITY OF G-11

Gump (1945) found G-11 to be relatively nontoxic to guinea pigs when administered orally. According to a study conducted by the Applied Research Laboratories (1952), the LD_{80} for guinea pigs is 280—300 mg/kg body weight and it was found mildly toxic when fed to rats in a concentration of 1: 5000 during a

TABLE 1

SOLUBILITY OF G-11 IN VARIOUS SOLVENTS.
DATA REPORTED BY GUMP (1945), CHISHOLM ET AL. (1950), GREGG AND ZOPF (1951) AND SINDAR CORPORATION (1952)

Solvent	Solubility in grams per 100 ml at 25°C.
Water	0.0012
Ethyl alcohol 10 %	0.013
20 %	0.02
50 %	0.15
70 %	0.94
100 %	50
Isopropyl alcohol	31.6
Propylene glycol	24.5
Acetone	191
Benzene	9.3
Toluene	5.6
Isopropyl myristate-palmitate	20
Methyl ethyl ketone	77
Tween 80 10 % aqueous solution	0.5
Tween 20 10 % „	0.5

G-11 dissolves in soap solutions and in dilute potassium and sodium hydroxide solutions. It is practically insoluble in mineral oils, glycerine, petrolatum and paraffin.

period of thirty days. Florestano (1949) established that the LD_{50} for mice is 80 mg/kg, while Nickerson (1952) obtained the values 166—209 mg/kg, with an average of 187 mg/kg.

It may hence be concluded that G-11 is practically nontoxic when taken orally. Gump (1945) concluded on the basis of animal experiments that an average adult would have to swallow at least eight full cakes (about 800 g) of germicidal soap containing 2 % of G-11 to be endangered (without considering the toxicity of the soap itself). Price and Bonnet (1948) established that G-11 was highly toxic to dogs when injected intravenously, a dose of 5 mg/kg giving rise to convulsions, intravascular clotting of blood, sudden respiratory cessation, and death within a few minutes. For this reason the latter authors warned against its use in the treatment of open wounds.

Traub *et al.* (1944, 1945) conducted a series of patch tests on over 600 subjects using 0.5 and 1.0 per cent G-11 in petrolatum and

found that the compound was non-irritating to the skin and was not a skin sensitizer. These subjects included persons with allergic diseases, such as asthma, hay fever, and eczema. In similar tests with 200 subjects, Udinsky (1945) found no one sensitive to G-11. Using a standardised method to compare the irritating properties of soaps, Schwartz (1950) found that a perfumed soap containing 2 per cent G-11 gave rise to less irritation than the soap alone. Similar results concerning the irritant properties of G-11 were also obtained by Pedersen and Perdrup (1952) in patch tests on eczema patients. Freeman and Young (1949) reported that soap containing G-11 caused no detrimental effects when used to cleanse wounds and burns. This was later confirmed by Best *et al.* (1951) by both animal experiments and clinical trials. Investigators of the South Shore Analytical and Research Laboratory Inc. (1952) established by means of animal experiments that a 2 per cent solution of the sodium salt of G-11 in 40 per cent aqueous propylene glycol did not produce eye irritation.

Despite the fact that soaps containing G-11 have been used for several years in various parts of the world, only relatively few cases of skin irritation have been reported. Seastone (1947), Seastone and Erickson (1949), Kraissl (1950) and Howorth (1950) have each described one case. Schnohr (1952) established that application of a 2 per cent solution of G-11 in 60 per cent alcohol for one hour in the disinfection of the field of operation resulted in a very pronounced local irritation of the skin. Patch tests conducted with the same solution gave positive reactions in 21 out of 24 patients. Cleland (1952) reported a case of dermatitis in a surgeon who used a soap containing 3 per cent G-11 with a brush, but in whom no dermatitis became evident when he used the same soap without the brush. Also Dietrich (1954) observed that skin irritation may result when a preparation (''Satinasept'') containing G-11 was employed together with a brush.

THE BACTERIOSTATIC AND BACTERICIDAL PROPERTIES OF G-11

G-11 is a powerful bacteriostatic agent. It is more effective against Gram-positive than against Gram-negative organisms. Its inhibition concentration against micrococci varies from 1: 10^6

to 1: 10×10^6 according to Seastone (1947) and from 1: 5×10^6 to 1: 8×10^6 according to Price and Bonnett (1948). Inhibition of the growth of coliform organisms requires a ten- to a hundredfold concentration (Seastone 1947). Florestano (1949) found that among 29 diphenylmethanes G-11 was the most effective against *Mycobacterium tuberculosis,* complete inhibition being attained with 0.063 mg per cent of G-11 in Proskauer and Beck synthetic broth.

G-11 possesses strong bactericidal properties. Its phenol coefficient against *Micrococcus pyogenes* var. *aureus* as determined at 37° C by the F.D.A. method has been reported to be approximately 125 (Gump 1945). It is considerably less effective against Gram-negative organisms such as *Escherichia coli* and *Salmonella typhosa;* for the latter the phenol coefficient has been found to be 20 (Glaser *et al.* 1951). Price and Bonnett (1948) reported that one part of G-11 in one million parts of 0.01 N sodium hydroxide killed suspensions of *Micrococcus pyogenes* var. *aureus* after 30 minutes exposure at room temperature, but the Sindar Corporation (1952) has found that a greater concentration (1: 25,000) is required to achieve the same result. Neither of these reports contains any figures on the effect on the test organisms of the alkaline solutions used as solvents. Lawrence and Erlandson (1953) have established that the spore-forming Gram-positive rods *Bacillus mesentericus* and *Bacillus subtilis* are definitely more sensitive than *Micrococcus pyogenes* var. *aureus* to G-11.

An outstanding property of G-11 is that it retains its bactericidal properties when dissolved in soap solutions. This has been ascribed to the fact that one of its phenolic hydroxyl groups reacts with soap solutions to form a monosalt while the other remains free. Berthet (1947) observed that the surface active agent "Aerosol OT" effects in a concentration of 1: 2000 a fourfold increase in the phenol coefficient of G-11.

Several investigators have shown that serum (Seastone 1947, Fahlberg *et al.* 1948, Dull *et al.* 1950, Florestano and Bahler 1953, and Lawrence and Erlandson 1953), serum globulin, egg albumin (Fahlberg *et al.* 1948, Lawrence and Erlandson 1953), and serum albumin (Lawrence and Erlandson 1953) strongly reduce the antibacterial activity of G-11. One per cent serum in broth containing G-11 is able to reduce the bacteriostatic action of the latter against

Micrococcus pyogenes var. *albus* about a hundredfold (Seastone 1947, and Fahlberg *et al.* 1948).

Although serum has thus been observed to suppress the activity of G-11, the Sindar Corporation (1952) reports that 0.1 per cent G-11 in 50 per cent serum kills *Micrococcus pyogenes* var. *aureus* within 24 hours, i.e. when the time of contact is sufficiently long.

Gregg and Zopf (1951) concluded that Tween 80 greatly enhances the bactericidal properties of G-11. However, several investigators have observed definite antagonism between non-ionic detergents and G-11 and / or related substances (Bolle and Mirimanoff 1950, Engler and Mirimanoff 1951, Bouchardy and Mirimanoff 1951, Bolle and Mirimanoff 1951, 1952, Lawrence and Erlandson 1953, and Erlandson and Lawrence 1953.) This has been assumed to be due to the free fatty acids present in the detergent (primarily oleic acid, Bolle and Mirimanoff 1950) or possibly due to chemical reaction between the antiseptic and detergent or to a change in the permeability of the cell membranes (Engler and Mirimanoff 1951). In order to explain the results of Gregg and Zopf, Bolle and Mirimanoff (1952) consider the possibility that the acid pH at which the former authors conducted their experiments is more effective against *Micrococcus pyogenes* var. *aureus* than the neutral or slightly alkaline pH of their own studies.

THE EFFECT OF G-11 ON THE BACTERIAL FLORA OF THE SKIN

In 1938 Price published a new method for the assessment of the bacterial flora of the hands, particularly for the evaluation of the effect of disinfectants on it. As a result of studies extending over nine years, Price divided the skin bacteria into transient and resident organisms. Transient organisms are contracted by contact and vary considerably both in number and kind depending on the conditions and are present in greater number in the uncovered skin areas, and under the fingernails. The resident organisms constitute a relatively stable flora, they increase by multiplication in situ and diminish through death, friction and the action of disinfectants. Price also introduced a new term "degerm" which he defined as follows: to degerm an object is to reduce, by any means, the number of microbes, pathogenic ar nonpathogenic, in or on it. He observed that after complete degerming the resident flora of the hands and forearms required a week or more to attain its former level. Under

clothing and other cover this process developed more rapidly; especially under rubber gloves the flora rapidly increased to its former level.

The transient flora can be easily removed. It may include numerous pathogens, which, however, frequently disappear spontaneously in a short time. Rebell *et al.* (1950) considered the main cause of this to be desiccation. Evans *et al.* (1950) assumed that the antibiotic properties of certain bacteria are responsible for the reduction of the number of bacterial species on the skin. As Price (1938) has found, certain pathogens may become resident whereupon their removal will be difficult, since washing, for instance, reduces the resident flora relatively slowly. Price has stressed the significance of this observation in surgical practice.

Lovell (1945) observed that in normal conditions the skin bacteria are embedded in the horny fat, in the crypts and crevices and in the hair follicles and sebaceous glands. Most of the transient organisms are located quite superficially while the resident organisms are hidden in the hair follicles and sebaceous glands and cannot be dislodged by scrubbing without damaging the skin. He also remarks that even despite thorough preoperative skin degermation, the resident organisms may rise to the skin surface and bring about wound infection.

Saarenmaa (1946) observed that a very abundant flora still remains on the skin after profuse perspiration and washing in the Finnish sauna (steam bath) even if it undergoes a distinct reduction during the bathing. The flora is mainly composed of micrococci, 0.2—0.3 per cent of which were pathogenic in his material. The criteria for pathogenicity he employed in his study were pigment production, haemolysis, plasma coagulation and mannite fermentation.

Attempts to prepare a germicidal soap before the introduction of G-11 were mostly unsuccessful, the results obtained in the degermation of the skin being primarily due to the physico-chemical action of the soap itself.

The first study of G-11 published in the medical journals was that of Traub *et al.* (1944). They, like most of the other investigators, employed a modification of Price's method and observed that when bar soap containing 2 per cent G-11 was regularly and solely used, the resident flora of the hands was decidedly reduced and remained at a very low level as long as the soap was used. On the basis of animal experiments, they concluded that the use of such a soap decreases the possibility of infection of superficial wounds. Further, these authors (1945), and also Reid *et al.* (1950), observed that G-11 is actually a bactericide and does not bring about only an apparent reduction in the bacterial flora of the skin, as has been observed to be the case with mercurials (Price 1939),

and quaternary ammonium compounds (Miller *et al.* 1943, Blank and Coolidge 1950a, Naumann 1952, and Canzonetti and Dalley 1952), but has also a similar action on pathogenic as well as non-pathogenic micrococci. Traub *et al.* (1944), Price and Bonnet (1948) as well as Blank and Coolidge (1950b) observed that an occasional washing with G-11 soap does not effect a greater reduction in the skin flora than a washing with ordinary soap, but that the regular use of the G-11 soap reduces the number of the bacteria, according to Price and Bonnet (1948), to about 5 per cent of the normal. The latter authors (1948) also noted that G-11 did not show any specific action on pathogenic micrococci.

Seastone (1947) found that a bacteriostatic concentration of G-11 against normal skin organisms was retained on the hands after liquid soap containing 1 per cent G-11 had been used once daily on five days of the week. He noted that prolonged lathering with plain soaps and rinsing with alcohol (see also Best *et al.* 1950) diminished the activity of G-11, presumably by dissolving it from the skin, and that relapses more than two days in the use of G-11 soap decreased its activity appreciably.

Clark *et al.* (1947) observed that the bacterial count on the hands decreased by one-half during one hour when rubber gloves were worn after washing with liquid soap containing 2 per cent G-11, while an approximately 100 per cent increase was noted for the controls. Fahlberg *et al.* (1948) established that the removal of the skin fats with acetone or ether strengthened and prolonged the bacteriostatic action of G-11 on the skin. G-11 was demonstrated on the skin even up to 2 days after three consecutive daily applications of a liquid soap containing 1 per cent G-11. Only one-tenth of the amount of G-11 was recovered from the skin after a similar application of a solid 2 per cent G-11 soap. Seastone and Erickson (1949) also noted a considerably greater reduction in the bacterial flora following the use of liquid soap than after the use of solid soap as a vehicle for G-11.

Employing a biological method, Nungester *et al.* (1949) and Thirlby and Nesbit (1949) compared a 3-minute brushless wash with bar soap containing 2 per cent G-11 with a routine 10-minute surgical two-brush scrub on five successive days and found that washing with G-11 soap was more effective after the second day, which indicates a cumulative action of G-11.

Dull *et al.* (1950) observed that a single 3-minute wash with liquid soap containing 0.24 per cent G-11 was at least as effective as a 10-minute scrub with a bland cake soap when the bacterial counts at the end of a 2-hour operation were compared. A single 3-minute wash with pHisoderm [1] containing 3 per cent G-11 corresponded to the immediate effect of a 10-minute scrub with bland cake soap. Artz *et al.* (1951), using a skin biopsy culture technique, stated that a 3-minute scrub with the combination pHisoderm and 3 per cent G-11 was as effective as a routine 10-minute surgical scrub.

Schnohr (1952, 1953) has on the basis of his studies stated that the combination of 3 per cent G-11 and pHisoderm (or a similar preparation, ''formula 106'') gives a more rapid degermation than a surgical scrub, and that this combination is more effective in rapid degermation than G-11 in liquid or solid soap, the latter combination being unsuitable for this purpose. The reason for the superiority of pHisoderm as a vehicle for G-11 is presumably that ''during cleansing with pHisoderm the skin is embrocated with fat which contains G-11'' (Schnohr, 1953). However, Blank and Coolidge (1950 b) did not observe any significant difference in the activity of G-11 when applied in three detergents (soap, dermolate, pHisoderm) provided the preparations were used daily and regularly, whereas according to Harrison and Cockcroft (1952) the reduction can be better maintained if the combination 3 per cent G-11 and pHisoderm is used.

When examining the resistance of micrococci to the action of G-11, Grubb and Wands (1952) observed that no variation in the resistance occurred during the seven weeks their subjects used soap containing G-11. A similar conclusion had already been drawn by Seastone and Erickson (1949) from their observation that the low bacterial flora on the hands of their subjects did not undergo any change during the 3—4 months they used G-11 soap. Price (1951), however, has noted that the bacterial florae on the hands of different persons vary in their susceptibility to G-11. He warns against

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[1] pHisoderm is a proprietary synthetic detergent (Fairchild Bros. & Foster, New York) whose pH value 5.5 is the same as that of the normal skin. It is not a primary irritant and it is hypoallergic (Guild 1945).

overrating the effectivity of G-11 and considers a three-minute scrub with soap and brush followed by an alcohol rinse the best rapid method of degerming the skin. Schnohr (1953) stresses the significance of individual differences in the disinfection of the skin of the hands, the texture of which is an important factor.

Only a few investigations on the effectivity of G-11 have been based on a study of clinical material. Fuller *et al.* (1948) observed that a 2 per cent G-11 soap reduced the incidence and severity of pyogenic skin infections. G-11 in an oil-in-water lotion base (Glaser *et al.* 1951) and in a synthetic detergent (Lipschutz and Fitti, 1953) has been found to be effective in preventing and clearing miliaria, diaper rash and impetigo in newborn and older infants. In a study of the relation between preoperative washing of the hands by surgeons and postoperative wound infections, Bowers (1949) analyzed 1500 "clean" and 430 "potentially dirty" cases and found that a 4-minute brushless wash with pHisoderm containing 3 per cent G-11 was at least as simple and reliable a procedure as any used previously. Freeman and Young (1949, 1950) reported that the use of the combination pHisoderm and 3 per cent G-11 in the preoperative preparation of the operation field and the hands of the surgeon reduced the frequency of wound infections from 8.2 to 2.1 per cent. It is, however, true that postoperative wound infection is associated with so many different factors that it is unwise to draw any definite conclusions about the value of an antiseptic in combatting such infections. After presenting favorable results obtained with G-11, Kraissl (1950), for instance, remarks that "a low incidence of wound infections is usually the reflection of a careful surgical staff rather than of an efficient preoperative skin antiseptic".

The authors referred to above comprise only a minority of the numerous investigators who have undertaken a study of the value of G-11 as a antiseptic. Almost all investigators have expressed the opinion that continued use of soap or detergents containing G-11 is able to effect an appreciable reduction in the bacterial flora of the skin and keep the latter at a low level (Allers 1950, Hufnagel *et al.* 1948, Blank *et al.* 1950, Murphy *et al.* 1951, Mirecki 1954).

THE PURPOSE OF THE INVESTIGATION

When the present bacteriological study of G-11 was begun in the Spring of 1952, an examination of the papers on the subject revealed that the investigations conducted *in vitro* that have dealt with the antibacterial activity of G-11 dissolved in aqueous soluions of surface active agents were relatively rare, although such solutions must be considered the proper environment for G-11. A surface active agent suitable for this purpose and one that was readily available in pure form was the synthetic detergent sodium lauryl sulphate. When searching in the published papers for data with which the results obtained with the combination of this detergent and G-11 could be compared, I came across the work of Bean and Berry (1950, 1951) which provided a plausible explanation of my own findings and gave cause to continue the study of the activity of G-11 in aqueous sodium lauryl sulphate solutions and to extend these studies to solutions of G-11 in aqueous potassium laurate.

The discrepancies in the results of Bolle and Mirimanoff (1950) and other investigators on one hand, and in those of Gregg and Zopf (1951), on the other, led me to investigate the effect of Tween 80 on the antibacterial activity of G-11.

Since preparations containing G-11 had been marketed in Finland from 1952, it was of interest to compare the degerming action of one of these preparations with the results published elsewhere and to investigate whether the degerming action of a soap containing G-11 changes when the content of antiseptic is decreased from 2 to 1 per cent.

The main subjects of study that evolved from the above considerations were the following:

1. To investigate the antibacterial activity of G-11 dissolved in aqueous solutions of sodium lauryl sulphate and potassium laurate.

2. To examine the effect of Tween 80 and serum on the antibacterial activity of G-11 and to compare at the same time the sensitivities of various bacterial species to G-11.

3. To study the effect of soap containing G-11 on the bacterial flora of the hands.

THE PRESENT STUDIES

BACTERIAL STRAINS, CULTURE MEDIA AND CHEMICALS

The bacterial strains employed in this study had been isolated at the Department of Medical Microbiology of the University of Turku from routine samples. The following species were represented: *Micrococcus pyogenes* var. *aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, and Salmonella paratyphi B,* 20 strains of each; *Micrococcus pyogenes* var. *albus, Streptococcus nonhemolyticus* and *Salmonella typhosa,* 6 strains of each.

Culture media. Agar medium: NaCl 2.5 g, peptone 10 g, agar 40 g, meat extract 2000 g and Na_2HPO_4 4 g.
Broth: NaCl 2.5 g, peptone 5 g, meat extract 1000 g.
Serum broth: the preceding broth medium to which 2 per cent sterile serum had been added.

Chemicals. G-11 (Hexachlorophene) and its potassium salt were obtained from L. Givaudan & Cie, S.A., Switzerland.
The sodium lauryl sulphate was a product of Eastman-Kodak Co., U.S.A.
The potassium laurate was prepared in the Institute of Physical Chemistry of the Åbo Akademi, Turku, Finland.
Tween 80 was obtained from George T. Gurr Ltd., London.
The 94 per cent ethanol was a commercial product of Oy. Alkoholiliike, Helsinki, Finland.

The soaps used in this study were manufactured by Turun Saippua Oy., Turku, Finland.

THE ANTIBACTERIAL ACTIVITY OF G-11 IN AQUEOUS SODIUM LAURYL SULPHATE SOLUTIONS

EXPERIMENTAL METHOD

Since G-11 itself is sparingly soluble in aqueous sodium lauryl sulphate solutions, the more soluble potassium salt was used instead despite its fairly high solubility in pure water (Fig. 1). An

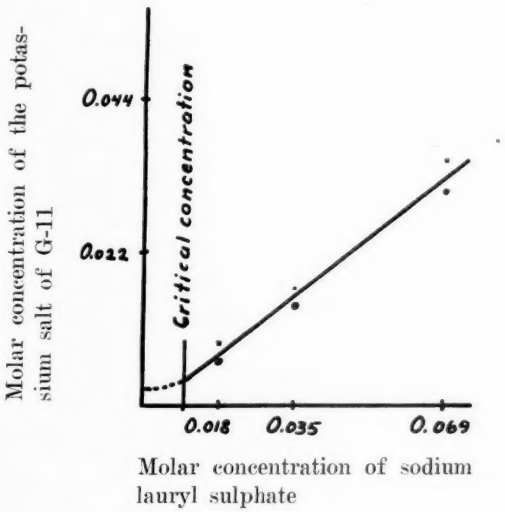

Fig. 1. The solubility of the potassium salt of G-11 in aqueous sodium lauryl sulphate solutions.

initial solution containing 0.07 mole of sodium lauryl sulphate and 0.03 mole of the potassium salt of G-11 per liter was prepared by dissolving the required amounts of these compounds in 10 ml of freshly boiled distilled water and allowing to cool to room temperature. Two series of dilutions were prepared from this solution. In the first case, the diluent was distilled water and in the other, a 0.07 M sodium lauryl sulphate solution. One milliliter volumes of the serial dilutions were transferred to small test tubes which were then immediately used in the tests.

Preliminary experiments showed that *Escherichia coli* and *Pseudomonas aeruginosa* were the most suitable test organisms.

Micrococcus pyogenes var. *aureus*, for example, was found to be too sensitive to the pure sodium lauryl sulphate solutions.

The bacteria were inoculated on agar plates, four strains to each plate, which were incubated 24 hours at 37° C. A thick aqueous suspension of the bacterial mass was then prepared by shaking five minutes by machine. By comparison with a Burroughs-Wellcome opacity tube series, a bacterial suspension containing approximately 1 million organisms per ml was prepared and 0.1 ml of this suspension was transferred with a sterile pipet to each of the tubes of the dilution series for the strain in question and mixed by vigorous shaking. Samples were taken with a platinum loop 4 mm in diameter 1, 2½, 5, 7½, 10, 15 and 30 minutes after the mixing was begun and inoculated into tubes containing 2 ml of serum broth. In each dilution series for each strain, there was one control tube that contained only the 0.07 M sodium lauryl sulphate solution. A sample was also taken from this tube after 30 minutes had elapsed. The inoculated tubes were incubated 48 hours at 37° C. The last tube in which growth was observed was noted. 20 *Escherichia coli* and 10 *Pseudomonas aeruginosa* strains were studied by this technique.

In the second set of experiments a saturated aqueous solution (about 1: 800) of the potassium salt of G-11 was prepared by boiling an excess of the salt in distilled water during five minutes. After the mixture had stood 24 hours at room temperature, it was centrifuged and the clear supernatant layer was employed in the experiments. The bactericidal properties of this solution and the 1 : 16 dilution were examined using the platinum loop method and 10 *Escherichia coli* strains.

In the third set the antibacterial activities of the initial solution, the 1 : 8 dilutions of both dilution series, and the control 0.07 M sodium lauryl sulphate solution were studied by the counting method. Two milliliters of these solutions were measured into tubes. Suspensions of each strain containing approximately 10,000 bacteria per ml were prepared as described above. From each suspension 0.1 ml was transferred with a sterile pipet to the each of the four tubes. One-tenth-ml samples were taken from the tubes immediately after the suspension was mixed and 2½, 5, 7½, 10, 15, and 30 minutes later, and added to 0.3 ml of serum on a Petri dish into which 15 ml of the melted agar medium at 44° C were then

poured, the dish being tilted to spread the agar. After the agar had set, the plates were placed in a + 37° incubator for 48 hours. Plates with unevenly distributed colonies were discarded. The number of colonies in three squares (1 sq. cm) on one diameter of the plate, one in the center and the other two at equal distances on each side, were counted. The number of bacteria per ml of each solution was then computed. This series was performed with the 20 *Escherichia coli* strains.

RESULTS

The results obtained by the platinum loop method are collected in Tables 2 and 3. All strains transferred from the control tubes containing 0.07 M sodium lauryl sulphate solution were found to

TABLE 2

THE BACTERICIDAL ACTIVITY OF G-11 IN AQUEOUS SODIUM LAURYL SULPHATE SOLUTIONS. THE MOLAR RATIO OF POTASSIUM SALT OF G-11 AND SODIUM LAURYL SULPHATE = 0.03/0.07 (0.43). THE STRAINS WERE TESTED WITH THE PLATINUM LOOP METHOD

Bacteria tested	Dilution	Number of strains that died during the period from							Mean death-time in minutes
		1—2 1/2 min.	2 1/2—5 min.	5—7 1/2 min.	7 1/2—10 min.	10—15 min.	15—30 min.	30— min.	
20 strains of Escherichia coli	1/1	1	9	5	4	1			5.71 ± 0.52 [1]
	1/2	1	4	6	6	3			7.21 ± 0.69
	1/4	4	2	6	5	3			6.66 ± 0.79
	1/8	2	8	2	6	2			6.17 ± 0.73
	1/16	2	7	7	4				5.43 ± 0.50
	1/32	2	1	8	6	3			7.36 ± 0.68
	1/64	2	2	4	6	4	2		9.17 ± 1.26
	1/128	1	2	1	5	5	6		12.84 ± 1.60
	1/256		1	1	1	3	11	3	> 16
	1/512					2	1	17	> 26
10 strains of Pseudomonas aeruginosa	1/1			1	6	2	1		10.62 ± 1.50
	1/2			1	3	4	2		12.75 ± 1.75
	1/4				2	4	4		15.70 ± 1.90
	1/8			1	2	1	6		17.12 ± 2.24
	1/16				1		9		21.13 ± 1.36
	1/32						6	4	> 23
	1/64							6	> 30

[1] Standard deviation of the mean.

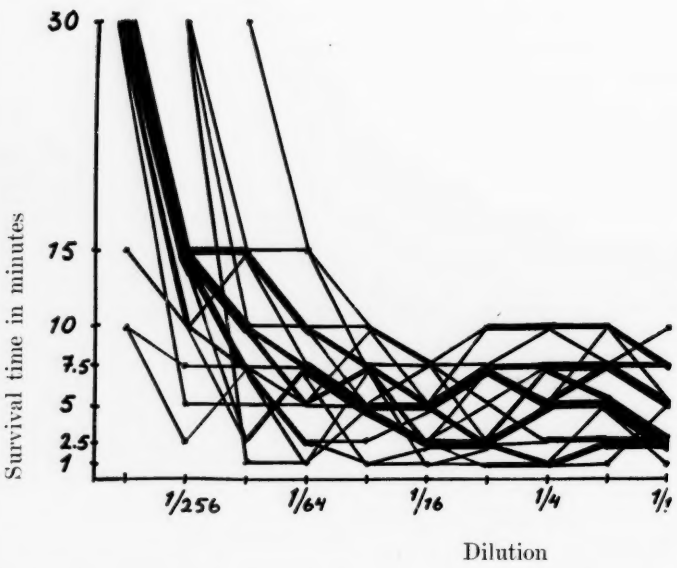

Fig. 2. The bactericidal activity of G-11 in aqueous sodium lauryl sulphate solutions with a constant molar ratio (0.43) of the potassium salt of G-11 to sodium lauryl sulphate. The concentrations in the initial solution were 0.03 M and 0.07 M, respectively. Twenty strains of Escherichia coli were tested by the platinum loop method. See Table 2.

grow after 30 minutes. In the tubes of the first series in which the molar ratio of the potassium salt of G-11 and sodium lauryl sulphate was held constant at 0.43 (i.e. the series in which the initial solution 0.03 molar in the potassium salt of G-11 and 0.07 molar in sodium lauryl sulphate was diluted with distilled water), the death-times for all *Escherichia coli* strains remained in the range from 1 to 15 minutes down to the dilution 1: 32 (Table 2 and Fig. 2). In more dilute solutions the death-time began to rise steeply, being less than 30 minutes for only 3 strains in the dilution 1: 512.

When the initial solution was diluted with the 0.07 M sodium lauryl sulphate solution, whereupon the concentration of the latter remained constant throughout and the molar ratio of the components decreased, the death-time increased more rapidly (Table 3). This time remained within the 1—15 minute range down to the dilution 1: 1.67 in respect of the potassium salt of G-11 for all strains examined; in the dilution 1: 20 it was less than 30 minutes

TABLE 3

THE BACTERICIDAL ACTIVITY OF G-11 IN AQUEOUS SODIUM LAURYL SULPHATE SOLUTIONS. THE MOLAR CONCENTRATION OF G-11 WAS REDUCED BY DILUTING THE INITIAL SOLUTION WITH THE 0.07 M SODIUM LAURYL SULPHATE SOLUTION. THE STRAINS WERE TESTED WITH THE PLATINUM LOOP METHOD

Bacteria tested	Dilution	Number of strains that died during the period from							Mean death-time in minutes
		1—2 1/2 min.	2 1/2—5 min.	5—7 1/2 min.	7 1/2—10 min.	10—15 min.	15—30 min.	30— min.	
20 strains of Escherichia coli	1/1	4	3	10	3				5.35 ± 0.54
	1/1.25	1	2	6	8	3			7.71 ± 0.64
	1/1.67	2	0	4	4	10			9.42 ± 0.83
	1/2.50			2	3	5	10		16.31 ± 1.49
	1/5				3	4	7	6	> 17
	1/10					2	5	7	> 23
	1/20						2	5	> 28
10 strains of Pseudomonas aeruginosa	1/1			1	4	4	1		11.37 ± 1.42
	1/1.25				1	6	3		15.12 ± 1.65
	1/1.67				1	2	7		19.12 ± 1.75
	1/2.50				1	1	5	3	> 17
	1/5					1	1	5	> 24
	1/10						2		> 25
	1/20							2	> 30

for only two strains. Thus in this series the 30-minute limit was attained with 18 strains before any strain had reached it in the water dilution series. It is thus seen that the bactericidal activity of the solution decreases rapidly with the molar ratio of potassium salt of G-11 to sodium lauryl sulphate (Fig. 3).

In the saturated aqueous solution of the potassium salt of G-11, the death-time of 10 strains of *Escherichia coli* averaged 6.0 ± 0.67 min. When this solution was diluted 1 : 16, the mean death-time was 25.0 ± 2.1 min. The concentration of the G-11 salt in the saturated solution was 0.0027 M.

Results similar to those obtained for the *Escherichia coli* strains were also obtained for the 10 *Pseudomonas aeruginosa* strains. The death-times were higher throughout for the latter strains. With a constant molar ratio, the death-time increased much more slowly on dilution with water than when the ratio was decreased by diluting with 0.07 M sodium lauryl sulphate.

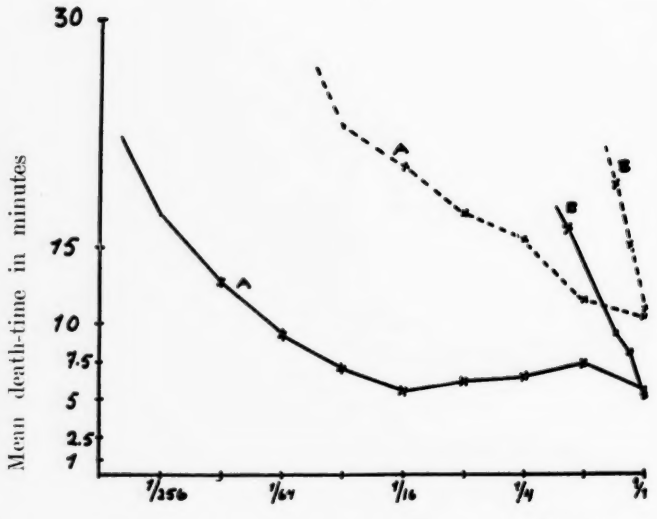

Fig. 3. The bactericidal activity of G-11 in aqueous sodium lauryl sulphate solutions. Mean death-times for 20 strains each of Escherichia coli and Pseudomonas aeruginosa tested with the platinum loop method.

A: Constant molar ratio (0.43) of the potassium salt of G-11 to sodium lauryl sulphate. The initial concentrations of the two components were 0.03 M and 0.07 M, respectively. Dilution with distilled water.
B: Molar ratio decreased by dilution with 0.07 M sodium lauryl sulphate.

———— Escherichia coli - - - - - - - - - Pseudomonas aeruginosa

See Table 3.

The variation of mean death-times in the dilution series is shown in Fig. 3. The averages for both test organisms are seen to deviate from each other already in the second dilution. When the molar ratio of potassium salt of G-11 to sodium lauryl sulphate is held constant, the death-time for *Escherichia coli* remains fairly constant down to the dilution 1: 32 and then increases. When the ratio is decreased by dilution with the sodium lauryl sulphate solution, the death-time begins to increase immediately. The initial points for the two curves for each bacterial species were determined separately. There is no significant difference between these

death-times which gives an idea of the reproducibility of the method employed.

In the series in which 20 *Escherichia coli* strains were studied by the counting method (Table 4 and Fig. 4.), it was established that the number of bacteria found living in the different solutions varied considerably already in the samples taken immediately after mixing. In the solution which was 0.03 M in the potassium salt of G-11 and 0.07 M in sodium lauryl sulphate, all strains were destroyed before the 15-minute samples were taken, 16 of them even before the 10-minute samples were taken. When this solution was diluted with seven parts of water, the molar ratio thus remaining constant, no growth was observed after 30 minutes in the case of 16 strains. In the second series in which dilution was effected by adding seven parts of the 0.07 M sodium lauryl sulphate solution whereupon the ratio changed to $^1/_8$ of the initial value, all strains were observed to grow even after 30 minutes exposure to the disinfectant solution. In Fig. 4, the average bacterial counts are plotted against the exposure time for 20 *Escherichia coli* strains. It will be seen that the antibacterial activity of

TABLE 4

THE ANTIBACTERIAL ACTIVITY OF G-11 IN AQUEOUS SODIUM LAURYL SULPHATE SOLUTIONS. 20 STRAINS OF ESCHERICHIA COLI TESTED WITH THE COUNTING METHOD

Solution	Molar concentration of sodium lauryl sulphate	Molar ratio of the potassium salt of G-11 and sodium lauryl sulphate
1	0.07	0.43
2	0.009	0.43
3	0.07	0.053
4	0.07	Control without G-11

Solution	Number of strains that died during the period from					
	0—2 $^1/_2$ min.	2 $^1/_2$—5 min.	5—7 $^1/_2$ min.	7 $^1/_2$—10 min.	10—15 min.	15—30 min.
1	1	2	5	8	4	—
2	—	—	—	—	7	9
3 and 4	—	—	—	—	—	—

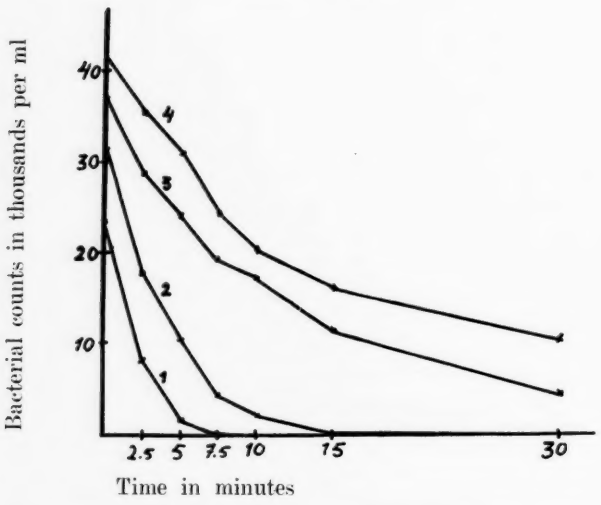

Fig. 4. The antibacterial activity of G-11 in aqueous sodium lauryl sulphate solutions. Mean bacterial counts for 20 strains of Escherichia coli. See Table 4.

the mixture is more dependent on the molar ratio of the components than on the absolute concentration of the potassium salt of G-11 in the solution. Reduction of the molar ratio thus effects a marked decrease in the antibacterial activity.

THE ANTIBACTERIAL ACTIVITY OF G-11 IN AQUEOUS POTASSIUM LAURATE SOLUTIONS

EXPERIMENTAL METHOD

The solubility of G-11 in aqueous potassium laurate solutions is shown in Fig. 6.

For this study, a dilution series was first prepared by diluting the 0.1 M potassium laurate solution with distilled water. A second solution was prepared that contained 0.1 mole of potassium laurate and 0.003 mole of G-11 per liter and this initial solution was diluted with distilled water to obtain a dilution series. A third solution was prepared which contained 0.06 mole of potassium laurate and 0.0024 mole of G-11 per liter. A series of dilutions was obtained from this by diluting with a 0.06 M potas-

sium laurate solution. All these solutions were prepared immediately before use.

The bactericidal properties of these solutions were studied by the platinum loop method with 20 *Escherichia coli* strains. One-tenth-ml volumes of a bacterial suspension of each strain containing approximately one million organisms prepared as described on p. 22 were added to 1 ml of each dilution in a test tube. Samples were taken from each tube after 1, 5, 10, 15, and 20 minutes and cultured as described on p. 22. The last tube in each series in which growth occurred was noted.

Both the first and the second dilution series were examined for their antibacterial action on 20 *Escherichia coli* strains using the counting method. The samples were taken after 1, 5, and 10 minutes.

RESULTS

The bactericidal activities of pure potassium laurate solutions as determined with the platinum loop method are shown in Table 5 and Fig. 5. It will be noted that the death-time increases as the potassium laurate concentration decreases.

In the water dilution series in which the molar ratio of G-11 and potassium laurate was held constant at 0.03, the death-times for all strains studied varied in a closely parallel manner. When the concentration was decreased from 0.1 M potassium laurate down to 0.001 M, a maximum followed by a minimum occurred

TABLE 5

THE BACTERICIDAL ACTIVITY OF POTASSIUM LAURATE SOLUTIONS. 20 ESCHERICHIA COLI STRAINS TESTED WITH THE PLATINUM LOOP METHOD

Molar concentration of potassium laurate	Number of strains that died during the period from						Mean death-time in minutes
	1—5 min.	5—10 min.	10—15 min.	15—20 min.	20—30 min.	30— min.	
0.10		3	8	4	5		15.88 ± 1.38
0.08			7	7	5	1	> 16
0.06			3	5	7	4	> 19
0.04				5	3	7	> 23
0.02					3	5	> 27

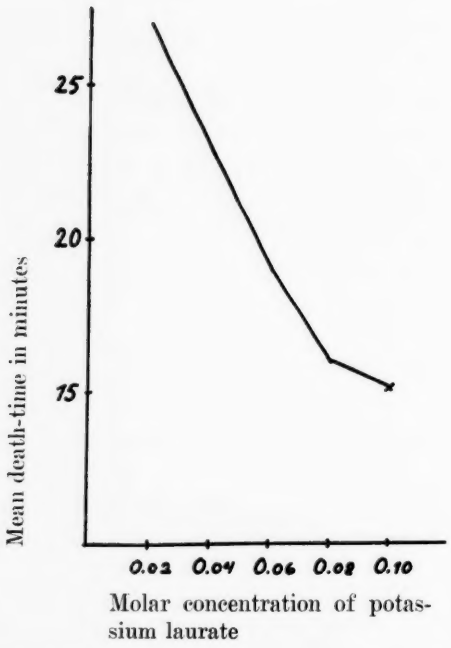

Fig. 5. The bactericidal activity of aqueous potassium laurate solutions. Mean death-times for 20 strains of Escherichia coli tested by the platinum loop method. See Table 5.

in the 0.05 — 0.01 M potassium laurate concentration range, after which the death-time rapidly increased over the 20-minute point. The data obtained for the 20 *Escherichia coli* strains are presented in Table 6 and Fig. 6. It may be noted that the critical concentration of potassium laurate is about 0.02 M; the mean death-time passes through a minimum at this concentration.

The results obtained when the concentration of G-11 was varied while the potassium laurate concentration was held constant at 0.06 M are seen in Table 7 and Fig. 7. The death-time increases gradually when the molar ratio of G-11 and potassium laurate becomes smaller; the bactericidal activity of the solution thus decreases continuously on dilution.

The mean death-times in the dilutions common to the two dilution series of G-11 (molar ratio = 0.03 and concentration of potassium laurate 0.06 M, Tables 6 & 7) show no significant difference which illustrates the reproducibility of the method.

TABLE 6

THE BACTERICIDAL ACTIVITY OF G-11 IN POTASSIUM LAURATE SOLUTIONS. THE MOLAR RATIO OF G-11 AND POTASSIUM LAURATE = 0.03. 20 STRAINS OF ESCHERICHIA COLI TESTED WITH PLATINUM LOOP METHOD

Molar concentration of potassium laurate	Number of strains that died during the period from					Mean death-time in minutes
	1—5 min.	5—10 min.	10—15 min.	15—20 min.	20— min.	
0.10	16	3	1			4.15 ± 0.57
0.08	15	3	2			4.63 ± 0.70
0.06	12	7	1			5.05 ± 0.62
0.05	7	11	1	1		6.67 ± 0.82
0.04	5	10	3	2		8.12 ± 1.00
0.03	1	7	7	4	1*	11.78 ± 1.10
0.02	11	3	4	2		7.03 ± 1.17
0.01	7	6	5	1	1*	8.40 ± 1.23
0.001					20	> 20

* Death-time taken to be 22.5 min.

The mean difference between the death-times for the 20 strains in the 0.03 and 0.02 M potassium laurate solutions is — 4.52 ± 1.80 min. (95 per cent confidence limits — 0.75 and — 8.29 min.).

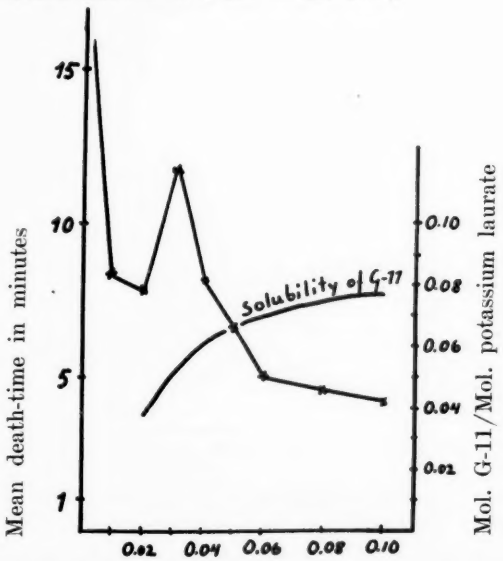

Fig. 6. **The bactericidal activity of G-11 in aqueous potassium laurate solutions with a constant molar ratio (0.03) of G-11 to potassium laurate. The initial concentrations of the two components were 0.003 M and 0.1 M, respectively. Mean death-times for 20 strains of Escherichia coli tested by the platinum loop method. See Table 6.**

TABLE 7

THE BACTERICIDAL ACTIVITY OF G-11 IN AQUEOUS POTASSIUM LAURATE SOLUTIONS. THE INITIAL SOLUTION (0.0024 M IN G-11 AND 0.06 M IN POTASSIUM LAURATE) WAS DILUTED WITH THE 0.06 M POTASSIUM LAURATE SOLUTION. 20 ESCHERICHIA COLI STRAINS TESTED WITH THE PLATINUM LOOP METHOD

Molar ratio of G-11 and potassium laurate	Number of strains that died during the period from					Mean death-time in minutes
	1—5 min.	5—10 min.	10—15 min.	15—20 min.	20— min.	
0.04	13	5	2			5.08 ± 0.72
0.03	13	3	4			5.58 ± 0.87
0.02	6	7	5	2		8.40 ± 1.07
0.01		4	4	5	7	> 13

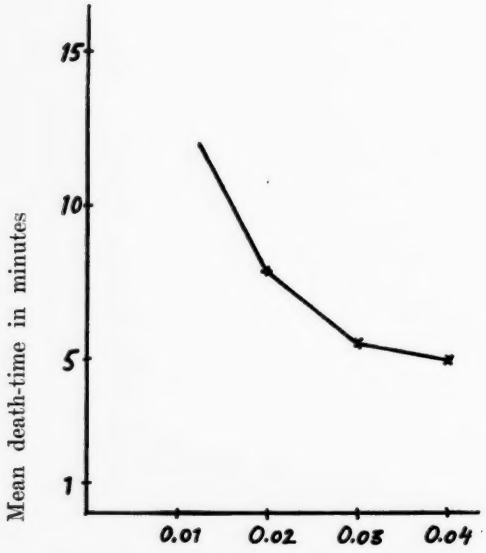

Fig. 7. The bactericidal activity of G-11 in an aqueous 0.06 M potassium laurate solution. Mean death-times for 20 strains of Escherichia coli tested by the platinum loop method. See Table 7.

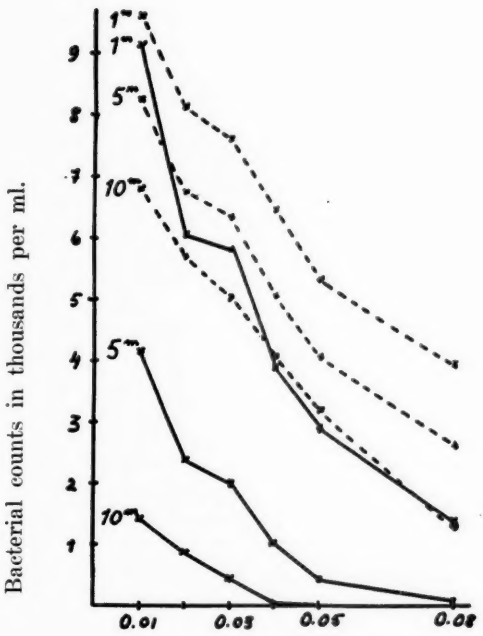

Fig. 8. The antibacterial activity of aqueous potassium laurate solutions with and without G-11. Mean bacterial counts for 20 strains of Escherichia coli. See Table 8.

———— Constant molar ratio (0.03) of G-11 and potassium laurate. The initial concentrations of the two components were 0.0024 M and 0.08 M, respectively.

- - - - - - - - Potassium laurate without G-11. The initial concentration was 0.08 M.

When the counting method was used to examine the effect of various concentrations of G-11 keeping the molar ratio of G-11 and potassium laurate constant at 0.03 (Table 8 and Fig. 8), it was found that when the potassium laurate concentration was decreased from 0.08 to 0.03 M, the antibacterial activity of the solution underwent a marked, progressive decrease, but did not decrease appreciably when the concentration of potassium laurate was diminished from 0.03 to 0.02 M. On further dilution, the anti-

bacterial activity again decreased fairly rapidly. This levelling-off in the 0.03—0.02 range is clearly evident in the mean bacterial counts after one minute, but no longer in the 5- and 10-minute counts.

When the action of potassium laurate without G-11 was studied, a similar variation in the 0.05—0.02 range was noted and most clearly in the 1- and 5-minute counts.

THE EFFECT OF TWEEN 80 ON THE ACTIVITY OF G-11

EXPERIMENTAL METHOD

The G-11 broth used in the bacteriostatic experiments was prepared by adding 0.1 ml of an ethanol solution of G-11 to 50 ml of broth. Two-ml volumes of this broth were added to test tubes and inoculated with one drop of a three-hour broth culture of the bacterial strain in question. The results were read after 48-hour incubation at 37° C. A control tube was included containing 2 ml of broth to which 0.1 ml of ethanol had been added per 50 ml of broth. The effect of Tween 80 was studied by adding known amounts of this substance to the G-11 broth.

For the tests by the counting method, a solution containing 100 mg of the potassium salt of G-11 in 100 ml of 0.001 N aqueous sodium hydroxide was prepared. This initial solution was covered with sterile paraffin oil immediately after its preparation. It was diluted before the tests with similarly stored 0.001 N sodium hydroxide. A suspension of bacteria containing about 10,000 bacteria per ml was prepared and from this 0.1-ml volumes were added to the test tubes of the dilution series. One-tenth-ml samples were taken immediately after mixing, and after 2½ and 5 minutes, and embedded in agar as described on p. 22. The colonies were counted after 48-hour incubation at 37° C. The effect of Tween 80 was studied by adding it to the G-11 solutions and determining the bacterial count as above. The pH values of the various solutions were measured with a potentiometer (Radiometer PHM21a.) The effect of Tween 80 on the bacteriostatic activity of G-11 was also studied using the paper disc method. For comparison, the effect of blood serum (sterile horse or bovine serum) was also examined by

TABLE 8

THE ANTIBACTERIAL ACTIVITY OF AQUEOUS POTASSIUM LAURATE SOLUTIONS WITH

TABLE 8

THE ANTIBACTERIAL ACTIVITY OF AQUEOUS POTASSIUM LAURATE SOLUTIONS WITH AND WITHOUT G-11. TWENTY STRAINS OF ESCHERICHIA COLI TESTED WITH THE COUNTING METHOD. BACTERIAL COUNTS IN THOUSANDS / ML

Solution	Time in min.	Mean bacterial counts for various molar concentrations of potassium laurate						Mean differences between bacterial counts for various molar concentrations of potassium laurate				
		0.01 M	0.02 M	0.03 M	0.04 M	0.05 M	0.08 M	0.01/0.02	0.02/0.03	0.03/0.04	0.04/0.05	0.05/0.08
Potassium laurate with G-11. Moles G-11/Moles potassium laurate = 0.03.	1	9.13 ± 0.35[1]	6.06 ± 0.37	5.82 ± 0.39	3.90 ± 0.30	2.90 ± 0.26	1.49 ± 0.27	3.01 ± 0.28[2]	0.26 ± 0.11	2.32 ± 0.28	0.93 ± 0.18	1.78 ± 0.16
	5	4.17 ± 0.39	2.40 ± 0.36	2.10 ± 0.36	1.07 ± 0.25	0.43 ± 0.10	0.18 ± 0.04	2.04 ± 0.15	0.45 ± 0.13	0.78 ± 0.33	0.71 ± 0.30	0.19 ± 0.04
	10	1.44 ± 0.34	0.89 ± 0.25	0.44 ± 0.18	0.07 ± 0.02	0.02 ± 0.01	—	0.49 ± 0.33	0.47 ± 0.28	0.41 ± 0.26	0.05 ± 0.02	—
Potassium laurate without G-11.	1	9.66 ± 0.32	8.28 ± 0.32	7.64 ± 0.32	6.48 ± 0.27	5.34 ± 0.23	3.95 ± 0.20	1.31 ± 0.13	0.76 ± 0.19	1.77 ± 0.25	1.11 ± 0.14	1.30 ± 0.17
	5	8.25 ± 0.24	6.75 ± 0.26	6.35 ± 0.25	5.08 ± 0.25	4.08 ± 0.18	2.62 ± 0.19	1.05 ± 0.13	0.52 ± 0.13	1.13 ± 0.21	0.99 ± 0.17	1.81 ± 0.22
	10	6.81 ± 0.25	5.73 ± 0.27	5.01 ± 0.21	4.03 ± 0.25	3.23 ± 0.22	1.37 ± 0.19	0.70 ± 0.11	0.35 ± 0.19	0.75 ± 0.11	0.82 ± 0.13	2.02 ± 0.32

[1] Standard deviation of the mean.

[2] Standard deviation of the mean difference. The mean differences were computed from the differences for two groups of 10 strains selected by means of a table of random numbers.

the same method. The paper discs 9 mm in diameter were stamped from the soft blotting paper. A solution of G-11 was prepared by dissolving G-11 in ethanol to give a concentration of 200 mg per ml. A series of solutions of G-11 was prepared from this by twofold dilution with ethanol and 0.0125-ml volumes of each dilution were added to the paper discs with the aid of micro-pipet. The discs were then dried at 37° C for one hour and stored at room temperature. Control paper discs were prepared in a similar manner by adding 0.0125 ml of alcohol containing no G-11.

The plates used in the studies contained 15 ml of the agar medium. The strains under study were cultured in 2 ml broth tubes for 3 hours at 37° C, after which the broth was poured on the agar plate and spread by tilting, the excess being then removed. The plates were then allowed to dry 2 hours at 37° C. Paper discs, 3 in number, containing different amounts of G-11, were placed on the agar surface and the plates incubated 24 hours at 37° C. The diameters of the inhibition zones were measured to the nearest millimeter. The Tween 80 and serum were mixed with the agar medium before the plates were poured.

Twenty *Micrococcus pyogenes* var. *aureus* strains were employed in the bacteriostatic test-tube and the paper disc studies and 10 strains of the same species in the studies by the counting method.

RESULTS

In the bacteriostatic tests conducted in test tubes, the growth of all the *Micrococcus pyogenes* var. *aureus* strains was inhibited by G-11 in a dilution of 1: 10^6; growth was retarded by G-11 in the dilution $1 : 2 \times 10^6$. All strains grew well when the dilution was $1 : 4 \times 10^6$. No inhibition was noted in the control tubes. When the effect of Tween 80 was examined, it was established that 2 of the 20 strains grew in the medium which contained G-11 in the dilution of 1: 100 000 and 0.01 per cent Tween 80, and all strains grew well when the Tween 80 content was 0.1 per cent. All strains grew in media containing G-11 in the dilutions $1: 2 \times 10^6$ and $1 : 5 \times 10^6$, and 0.01, 0.001, and 0.0001 per cent Tween 80.

The results of the paper disc tests on the effect of Tween 80 and serum on the bacteriostatic activity of G-11 on 20 strains of *Micrococcus pyogenes* var. *aureus* are shown in Table 9. The addition

TABLE 9

THE EFFECT OF TWEEN 80 AND SERUM ON THE BACTERIOSTATIC ACTIVITY OF G-11 AS DETERMINED BY THE PAPER DISC METHOD. TEST ORGANISM: MICROCOCCUS PYOGENES VAR. AUREUS (20 STRAINS). DIAMETERS OF INHIBITION ZONES IN MM. DIAMETER OF PAPER DISCS 9 MM

Medium contained	Concentration of G-11 in mg per ml.					
	200	100	50	25	12.5	6.25
No Tween 80 or serum	16.15 ± 0.26	16.15 ± 0.26	16.15 ± 0.26	16.10 ± 0.26	15.70 ± 0.22	15.55 ± 0.23
1 % Tween 80	10.85 ± 0.08	10.80 ± 0.09	10.35 ± 0.13	9.35 ± 0.13	9.29 ± 0.10	9.10 ± 0.07
2 % Tween 80	10.25 ± 0.10	10.15 ± 0.08	9.75 ± 0.12	9.10 ± 0.07	9.0	9.0
1 % serum	13.55 ± 0.11	13.55 ± 0.11	13.55 ± 0.11	13.55 ± 0.12	13.50 ± 0.12	13.45 ± 0.11
2 % serum	12.70 ± 0.13	12.70 ± 0.13	12.60 ± 0.11	12.25 ± 0.12	12.10 ± 0.12	12.00 ± 0.13

of Tween 80 is seen to decrease the diameter of the inhibition zone more than the addition of an equal volume of serum.

The effect of Tween 80 on the antibacterial properties of G-11 dissolved in aqueous 0.001 N sodium hydroxide is seen in Table 10. Tween 80 is observed to inhibit the activity of G-11 even in a

TABLE 10

THE EFFECT OF TWEEN 80 ON THE ACTIVITY OF G-11 IN 0.001 N NaOH SOLUTION. MEAN VALUES OF TESTS WITH 10 STRAINS OF MICROCOCCUS PYOGENES VAR. AUREUS.
THE BACTERIAL COUNTS ARE GIVEN IN THOUSANDS PER ML

Solution	Mean bacterial counts at		
	0 min.	2 1/2 min.	5 min.
0.001 N NaOH	27.80±1.40	18.20±0.77	12.60±0.71
G-11 1/100	3.29±0.90	0	0
G-11 1/1000	12.80±1.00	5.15±1.04	4.02±0.72
G-11 1/1000 + Tween 80 1%	17.77±0.59	12.47±0.47	7.04±0.63
G-11 1/1000 + Tween 80 0.01 %	6.33±0.47	3.52±0.57	1.11±0.36

concentration of 0.01 per cent. The pH values of the solutions studied varied in the range 10.2—10.8, which variation can hardly be considered responsible for the observed variation in the antibacterial activity in the presence of Tween 80.

THE SUSCEPTIBILITY OF SOME BACTERIAL SPECIES TO G-11 AS DETERMINED BY THE PAPER DISC METHOD

EXPERIMENTAL METHOD

The effect of G-11 on the other bacterial species mentioned on p. 20 was studied by the paper disc method as described in the preceding chapter. For comparison, similar tests were conducted with phenol using 20 *Micrococcus pyogenes* var. *aureus* strains; the initial phenol solution containing 90 g of phenol in 10 g of distilled water [1] was diluted by twofold dilution to obtain a series of phenol solutions for the tests. The paper discs were made as described in the preceding chapter and were always used on the next day since it was established in preliminary experiments that the inhibition

[1] Phenolum liquefactum. (Pharmacopoea Fennica, Ed. VI. Helsinki 1937.)

TABLE 11

SUSCEPTIBILITY OF VARIOUS BACTERIA TO G-11 AS DETERMINED BY THE PAPER DISC METHOD. THE DIAMETERS OF THE INHIBITION ZONES ARE IN MM. DIAMETER OF THE PAPER DISCS 9 MM

Bacteria	Concentration of G-11 in mg per ml.					
	200	100	50	25	12.5	6.25
Micrococcus pyogenes var. aureus 20 strains	16.15 ± 0.26	16.15 ± 0.26	16.15 ± 0.26	16.10 ± 0.26	15.70 ± 0.22	15.55 ± 0.23
Escherichia coli 20 strains	11.25 ± 0.14	11.15 ± 0.13	10.95 ± 0.09	10.70 ± 0.14	10.15 ± 0.17	9.95 ± 0.19
Pseudomonas aeruginosa 20 strains	11.55 ± 0.17	11.05 ± 0.22	10.95 ± 0.21	10.30 ± 0.21	9.75 ± 0.19	9.35 ± 0.18
Proteus vulgaris 20 strains	13.15 ± 0.24	13.05 ± 0.28	12.70 ± 0.29	12.30 ± 0.31	12.05 ± 0.33	11.60 ± 0.33
Salmonella paratyphi B 20 strains	11.85 ± 0.08	11.85 ± 0.08	11.80 ± 0.09	11.35 ± 0.16	11.00 ± 0.12	10.80 ± 0.12
Salmonella typhosa 6 strains	13.17 ± 0.40	13.17 ± 0.40	13.17 ± 0.40	12.87 ± 0.40	12.13 ± 0.31	12.00 ± 0.36
Micrococcus pyogenes var. albus 6 strains	18.50 ± 0.56	18.33 ± 0.49	18.33 ± 0.49	18.13 ± 0.54	18.30 ± 0.54	18.13 ± 0.54
Streptococcus nonhemolyticus 6 strains	15.00 ± 0.26	15.00 ± 0.26	15.00 ± 0.26	15.00 ± 0.26	14.33 ± 0.21	14.33 ± 0.21

zones decreased in diameter with the age of phenol-containing discs. The paper discs that contained G-11 did not lose their inhibitory power even after one week's storage at room temperature.

RESULTS

The effect of G-11 on the bacteria studied is shown in Table 11 and that of phenol on *Micrococcus pyogenes* var. *aureus* in Table 12. It will be observed that in the concentration range of G-11 investigated, the zone of inhibition did not undergo any greater reduction in diameter, while the zone obtained with phenol diminished rapidly with the concentration of the latter. An inspection of the results for the different bacteria reveals that the Gram-positive cocci are more sensitive to G-11 than the Gram-negative rods.

TABLE 12

THE SUSCEPTIBILITY OF 20 MICROCOCCUS PYOGENES VAR. AUREUS STRAINS TO PHENOL AS DETERMINED BY THE PAPER DISC METHOD. THE DIAMETERS OF THE ZONES OF INHIBITION ARE GIVEN IN MM. DIAMETER OF THE PAPER DISCS 9 MM

Phenol concentration in mg/ml	9000	4500	2250
Mean diameter of inhibition zone	27.15 ± 0.97	16.55 ± 0.50	9.0

DEGERMATION OF THE HANDS BY SOAP CONTAINING G-11

EXPERIMENTAL METHOD

Since it could be assumed that the hands of persons working in an operating theater, who perform a surgical scrub several times a day, carry a low bacterial flora, the flora of these persons was taken as a basis of comparison in the study of the effect of G-11 soap on the bacterial flora of the hands of persons engaged in the surgical ward. The procedure followed was a modification of that developed by Price (1938). Each subject washed her hands up to the wrists with soap containing no G-11 in succession in two basins

previously rinsed with hot water, one minute in each, with 30 seconds for lathering and 30 seconds for rinsing. The time was measured with a stop watch. Immediately after, 0.1-ml samples were taken with a sterile pipet from each basin and cultured; the colonies were counted as described in connection with the counting method.

The washing procedure was performed by 9 operating theater nurses and by 13 nurses from the surgical ward. Soap cakes containing 2 per cent G-11 were then distributed to the surgical ward and to the members of the second group who participated in the initial washing tests and who were all requested to use only these soaps both at home and at work. All other soaps were removed from the surgical ward. After one week the bacterial flora on the hands of the subjects was again determined by the above procedure. None of the subjects had previously used soap containing G-11.

At the same time washing tests were conducted with 11 operating theater nurses. Immediately on arriving in the morning, each subject performed the routine surgical scrub with ordinary soap and brush followed by an alcohol rinse. After this the subjects put on rubber gloves for one hour during which they performed their usual duties. The bacterial flora on their hands were then examined by the single basin method with a one-minute washing period. On the next morning the same procedure was carried out by 12 operating theater nurses, some of whom had been in the group of the preceding day. This time, instead of performing the surgical scrub, the subjects washed their hands during 3 minutes with soap containing 2 per cent G-11 without using the brush or rinsing with alcohol. The subjects had not previously used soaps containing G-11.

When the effect of decreasing the G-11 content of the soap from 2 per cent to 1 per cent on the degermation was later studied, soap products containing G-11 had already come into general use. For this reason, all soaps containing G-11 were removed from the laboratory where the subjects who were to participate in these tests were employed, and soaps containing no G-11 were distributed to this laboratory and also to the persons employed there, all these persons being requested to avoid the use of G-11 soaps altogether during the next two weeks. After this time, the bacterial florae on the hands of the subjects were evaluated twice

at an interval of three days. As it was immediately established that all had a relatively high transient flora, the following washing procedure was followed. The hands were washed in basin 1 with soap containing no G-11 two minutes alternating 30-second periods of lathering and rinsing. In basin 2, the subjects rinsed their hands one minute and applied soap lather for one minute after the rinsing. Rinsing followed in basin 3 during one minute. At all stages the hands were washed up to the wrists. Bacterial samples were taken and cultured similarly as described above (p. 41). The 14 participants were then divided into two groups of seven, and soap containing 1 per cent G-11 was given to one group and soap containing 2 per cent G-11 to the other group for use both at work and at home so that those working in the same room used the same soap. All were requested to use the soaps distributed to them and to avoid all other soaps. The bacterial flora on their hands were examined after 4, 7 and 14 days.

In all these washing experiments, a control sample (0.1 ml) was cultured from each basin before the washing procedure; in no case did the bacterial count exceed 12,000 bacteria per liter.

RESULTS

The results of the series of tests performed with the nurses of the surgical ward and operating theater are collected in Tables 13, 14 and 15. The total initial bacterial count for those working in

TABLE 13

BACTERIAL COUNTS IN MILLIONS/1000 ML IH HAND WASHINGS WITH ORDINARY SOAP. 9 NURSES FROM AN OPERATING THEATER STAFF

Subject	Basin		
	1	2	1 + 2
1	0.38	0.73	1.11
2	0.26	0.05	0.31
3	0.38	0.31	0.69
4	1.25	0.28	1.53
5	0.31	0.25	0.56
6	0.23	0.08	0.31
7	0.07	0.01	0.08
8	0.05	0.01	0.06
9	1.21	1.24	2.45
Mean	0.46	0.33	0.79 ± 0.26

TABLE 14

BACTERIAL COUNTS IN MILLIONS / 1000 ML IN HAND WASHINGS OF 13 NURSES FROM THE SURGICAL WARDS BEFORE AND AFTER USING SOAP CONTAINING 2 PER CENT G-11 FOR ONE WEEK

Subject	Before using G-11 soap			After one week of using G-11 soap			
	Basin			Basin			Decrease in total flora per cent
	1	2	1 + 2	1	2	1 + 2	
1	7.15	0.90	8.05	0.15	0.23	0.38	95
2	3.45	1.80	5.25	0.13	0.01	0.14	97
3	1.80	0.38	2.18	0.29	0.05	0.34	82
4	2.61	0.58	3.19	2.05	0.24	2.29	28
5	1.28	0.38	1.66	0.09	0.07	0.16	91
6	3.20	0.89	4.09	0.07	0.01	0.08	98
7	3.08	1.62	3.70	0.17	0.05	0.23	94
8	1.86	0.57	2.43	0.06	0.03	0.09	96
9	2.56	1.21	3.77	0.08	0.08	0.16	96
10	1.15	0.44	1.59	0.38	0.18	0.56	64
11	0.19	0.03	0.22	0.05	0.004	0.054	77
12	0.84	0.26	1.10	0.18	0.06	0.24	78
13	0.41	0.14	0.55	0.06	0.04	0.10	82
Mean	2.20	0.70	2.90 ± 0.53	0.30	0.08	0.37 ± 0.16	83 ± 5.4

TABLE 15

BACTERIAL COUNTS IN MILLIONS / 1000 ML IN HAND WASHINGS OF NURSES FROM AN OPERATING THEATER STAFF AFTER GLOVES HAD BEEN WORN 1 HR AFTER THE SCRUB

Subject	After routine surgical scrub	After 3-min. brushless scrub with 2 % G-11 soap
2		0.16
3	0.50	
6	0.44	0.18
7	0.01	0.04
8	0.01	0.16
9	0.06	0.06
10	0.09	0.03
11	0.12	0.08
12	0.02	
13	0.04	
14	0.07	0.07
15	0.12	
16		0.04
17		0.41
18		0.12
19		0.27
Mean	0.13 ± 0.05	0.13 ± 0.03

the surgical ward varied from 0.55 to 8.05 millions per liter with an average of 2.9 ± 0.53 millions per liter. The count for those from the operating theater was considerably lower, from 0.06 to 2.45 millions per liter with an average of 0.79 ± 0.26 millions per liter. After using soap containing 2 per cent G-11 for one week, the count for the former group had decreased 28—98 per cent, average decrease about 83 ± 5.4 per cent, to the level 0.37 ± 0.16 million per liter, which is in the range for the normal flora of the operating theater nurses. In the case of 7 of the 13 nurses degermation amounted to 85 per cent, and in only two cases was the degermation less than 75 per cent. No significant differences were observed in the bacterial flora after wearing gloves for one hour when the operating theater nurses performed the surgical scrub on the first day and washed with soap containing G-11 on the second (Table 15).

In the two groups that used soaps containing 1 and 2 per cent G-11, a marked decrease in the bacterial flora occurred during the tests (Table 16). The decrease in the bacterial flora was in general much larger during the first week, but some reduction occurred in most cases also during the second week. The soap containing 2 per cent G-11 cannot be said to be any more effective than the soap with one per cent G-11. The results of the washing tests show the cumulative action of G-11. The average decrease in the total flora did not exceed 85 per cent in either of the groups.

TABLE 16

TABLE 16

THE EFFECT OF SOAP CONTAINING 1 AND 2 % G-11 ON THE BACTERIAL FLORA OF THE HANDS. BACTERIAL COUNTS IN MILLIONS / 1000 ML

Group	Subject	Day																Decrease in total bacterial flora during 14 days (%)
		Initial washings [1]				4th				7th				14th				
		Basin				Basin				Basin				Basin				Basin
		1	2	3	1+2+3	1	2	3	1+2+3	1	2	3	1+2+3	1	2	3	1+2+3	1+2+3
I (1 % G-11 soap)	1	5.27	0.17	3.50	8.94	3.40	0.12	2.35	5.87	0.59	0.16	0.29	1.04	3.00	0.32	2.10	5.42	40
	2	3.20	0.60	2.33	5.13	3.40	0.29	2.54	6.23	3.00	0.19	0.62	3.81	2.55	0.20	0.41	3.16	41
	3	3.88	0.20	1.49	5.57	0.69	0.11	0.20	1.00	0.56	0.16	0.26	0.98	0.31	0.12	0.22	0.65	88
	4	2.98	0.56	2.20	5.74	2.74	0.23	2.34	5.31	0.29	0.12	0.19	0.60	0.22	0.12	0.14	0.48	92
	5	3.93	1.58	3.28	8.79	3.00	0.48	2.10	5.58	2.75	0.39	2.10	5.24	2.50	0.25	0.46	3.22	63
	6	5.08	1.34	3.13	9.55	2.55	0.17	2.10	2.92	0.30	0.19	0.24	0.73	2.50	0.12	2.10	4.72	51
	7	4.13	1.31	3.05	8.49	3.00	0.12	2.10	5.32	3.00	0.19	2.35	3.54	2.50	0.13	0.26	2.89	66
II (2 % G-11 soap)	8	3.70	2.75	3.03	9.48	2.55	0.26	2.10	4.91	0.36	0.19	0.26	0.81	0.24	0.10	0.20	0.54	94
	9	6.63	0.23	4.88	11.74	2.55	0.35	1.06	3.96	3.00	0.26	2.10	5.36	3.00	0.21	1.18	4.59	61
	10	6.13	1.60	4.15	11.88	3.40	0.22	3.00	6.62	3.00	0.36	2.35	5.71	3.40	0.12	0.14	3.65	69
	11	5.80	1.58	3.60	10.98	3.00	0.12	2.10	5.22	0.78	0.21	0.36	1.35	0.52	0.19	0.28	0.99	91
	12	4.90	0.23	3.63	8.76	2.55	0.12	2.10	4.77	0.36	0.12	0.20	0.68	0.59	0.13	0.16	0.88	90
	13	4.45	0.21	3.18	7.84	3.40	0.20	2.75	6.35	0.64	0.23	0.49	1.36	0.32	0.12	0.20	0.64	92
	14	2.13	0.14	0.52	2.79	2.35	0.06	0.14	2.55	2.54	0.31	2.10	4.95	2.10	0.29	0.40	2.68	4
Mean	I	4.07	0.82	2.71	7.60	2.68	0.21	1.96	4.85	1.50	0.20	0.86	2.56	1.94	0.18	0.81	2.93	63 ± 8.1
	II	4.82	0.96	3.28	9.06	2.83	0.19	1.90	4.53	1.51	0.24	1.12	2.87	1.48	0.17	0.36	2.01	72 ± 13.0

[1] Mean counts for two washings at an interval of three days.

DISCUSSION

Tobie and Orr (1944) have drawn attention to the possibility that a sampling error may result from the variation in the size of the drop that remains on a platinum loop when samples are taken from a solution of an antiseptic that contains a surface-active compound. They suggest that a pipet be used to take the samples. This factor is of importance when phenol coefficients are being evaluated in a comparison of the bactericidal activities of various antiseptics. In the present study, however, the bactericidal action of various combinations of the same components have been compared. Thus, above the critical micelle concentration of the association colloid, the error caused by the different surface tensions of the solutions cannot have any appreciable effect on the results (see p. 8). In the counting method used as a control, the bactericidal effect and the relatively weaker bacteriostatic effect of G-11 are superimposed in the agar media, even when the medium contains serum.

When the antibacterial activity of the potassium salt of G-11 dissolved in sodium lauryl sulphate solutions is evaluated, it is necessary to take into account the solubility of the potassium salt in water. The saturated aqueous solution of the potassium salt of G-11 possesses a considerable bactericidal activity, and hence this dissolved part may play some part in determining the results of the tests. When the effect of the acid form of G-11 dissolved in potassium laurate solutions was studied, the solubility of the antibacterial agent in water was so slight (see Table 1) that it could not have had any practical influence on the results.

Bean and Berry (1950) came to the conclusion that the solubility of the sparingly water-soluble benzylchlorophenol in aqueous potassium laurate solutions is connected with the micelle formation by the association colloid in such a manner that the solubility attains

its maximum when the micelles are fully developed. In their papers (1948, 1951, 1953) on the bactericidal activity of benzylchlorophenol and chloroxylenol in aqueous potassium laurate solutions, they stated that "the bactericidal activity of water-insoluble phenols solubilized by aqueous soap solutions is a function of the concentration of the phenol within the micelles of the soap rather than the concentration of the phenol in the solution as a whole." The results obtained in the present study on the antibacterial activity of the potassium salt of G-11 in aqueous sodium lauryl sulphate solutions are in agreement with the above statement. The curve of the mean death-time of *Escherichia coli* in aqueous solutions containing G-11 and potassium laurate in the molar ratio 0.03 (Fig. 6) is similar to the mean death-time curve for *Escherichia coli* and *Pseudomonas aeruginosa* in solutions containing benzylchlorophenol or chloroxylenol and potassium laurate in constant molar ratio in the investigations of Bean and Berry (1951, 1953). Thus, in accordance with their theory, a decrease in the concentration of G-11 in the potassium laurate micelles is accompanied by a decrease in the bactericidal activity of the solution. The increase in the bactericidal activity in the higher laurate concentrations when the ratio of benzylchlorophenol to soap was held constant, Bean and Berry assume to be possibly due to a gradual increase in the number of micelles of maximal size containing the same concentration of the bactericide. This explanation may be extended to the present results if in addition it is noted that the bactericidal activity may increase because the laurate concentration increases and also because the alkalinity becomes greater.

The mean bacterial count curve (Fig. 8) obtained after 1-minute exposure of *Escherichia coli* to G-11 in potassium laurate solution (molar ratio 0.03) corroborates the results obtained with the platinum loop method. The slight levelling-off of the mean count curve in potassium laurate solutions containing no G-11 between 0.02 and 0.05 M brings up the question whether micelle formation itself has some effect on the antibacterial activity of the laurate solutions. It is unfortunate that Bean and Berry did not find it possible to extend their experiments to laurate concentrations below 0.08 M.

It is possible that clumping of bacteria, which must always be taken into account when using the counting method, may have

influenced the results obtained. On the other hand, many phenomena occur in the range of micelle formation that are not even well understood from the chemical viewpoint, and hence much more work is necessary before the above aspects of the effect of antiseptics in association colloid solutions can be elucidated.

The present results on the effect of Tween 80 on the antibacterial properties of G-11 are in accordance with the findings of Bolle and Mirimanoff (1950) and others. Frobisher (1927) found that the addition of a suitable amount of sodium oleate to a phenol or hexylresorcinol solution effected an increase in the bactericidal activity of the latter, while an excess of sodium oleate reduced the activity. In none of the concentrations studied was Tween 80 observed to reinforce the bacteriostatic activity of G-11. The inhibition by Tween 80 may also be explained by assuming that being a micelle forming substance Tween 80 incorporates the molecules of G-11 in its micelles and thus reduces the concentration of G-11 per unit area of the surface of the bacteria. The present results show that Tween 80 has a stronger inhibitory effect on G-11 than serum, which is in accordance with observations made by Lawrence and Erlandson (1953).

A decrease in the amount of antiseptic in the paper discs was found to have a different effect on the inhibition zones of G-11 and phenol. Hoffman and Phillips (1950) observed a similar discrepancy between the results for phenol, pentachlorophenol and triiodophenol. According to these authors, differences in the rates of diffusion may play a part in the results obtained when the effects of various antiseptics are compared by the paper disc method.

As Price (1938) observed when he developed his method for evaluating of the bacterial flora of the hands, every person has his own characteristic flora. It is hence readily understood, as Schnohr (1953) has pointed out, that the effect of an antiseptic on the flora varies with the individual. Appreciable differences are also shown by the present results on the variation of the bacterial flora on the hands of subjects when they used soap containing G-11.

Cade (1950) described in detail the method he used to evaluate the degerming action of G-11. According to his opinion, at least six subjects should participate in the tests. A reduction of 85 per cent or more in the resident flora he regards a good degermation.

The method used in the present study deviated from that of Cade, but his criterion for an efficient degermation may also be adopted when evaluating the results of tests relating to the decrease in total flora. The average reduction in the bacterial flora of the hands effected by G-11 as found in the present study is smaller than that reported by most other workers, which is generally over 90 per cent. The observations on the effect of a single brushless scrub with soap containing 2 per cent G-11 conform with results of Traub *et al.* (1944), Price and Bonnet (1948) and Blank and Coolidge (1950 b) (see p. 15).

SUMMARY AND CONCLUSIONS

The bactericidal activity of the potassium salt of G-11 dissolved in aqueous sodium lauryl sulphate solutions has been studied by the platinum loop method using as test organisms 20 *Escherichia coli* strains and 10 *Pseudomonas aeruginosa* strains and by the counting method using 20 *Escherichia coli* strains. The activity of G-11 in aqueous potassium laurate solutions was also studied using the same methods, 20 *Escherichia coli* strains being employed as test organisms.

The effect of Tween 80 on the bacteriostatic activity of G-11 was examined in broth in the case of 20 *Micrococcus pyogenes* var. *aureus* strains. The effect of Tween 80 added to the media was compared with that of serum by the paper disc method. The counting method was employed to determine the effect of Tween 80 on the action of G-11 on 10 *Micrococcus pyogenes* var. *aureus* strains.

The susceptibility to G-11 of 20 strains each of *Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Salmonella paratyphi B* and *Micrococcus pyogenes* var. *aureus,* and of 6 strains each of *Salmonella typhosa, Streptococcus nonhemolyticus* and *Micrococcus pyogenes* var. *albus,* and the susceptibility to phenol of 20 strains of *Micrococcus pyogenes* var. *aureus* was evaluated by the paper disc method.

The effect of soap containing 2 per cent G-11 on the bacterial flora on the hands of 13 nurses from the surgical ward was studied and the resulting flora compared with the normal flora on the hands of 9 nurses engaged in the operating theater. A single washing with soap containing 2 per cent G-11 without a brush was compared with the routine surgical scrub in a group of 16 operating theater nurses. The effect of soaps containing 1 and 2 per cent G-11 was compared in two groups of seven subjects who

were employed in the bacteriological laboratory. In the studies on the degerming effect of soap containing G-11, three modifications of the Price method were employed.

The following conclusions were drawn on the basis of the results obtained:

The antibacterial action of G-11 in aqueous solutions of sodium lauryl sulphate and potassium laurate is more dependent on the molar ratio of G-11 to the association colloid than on the total concentration of G-11 in the solution, which in accordance with the theory of Bean and Berry implies that the antibacterial activity of the solution is primarily determined by the concentration of G-11 in the association colloid micelles. Tween 80 has a marked inhibitory effect on the antibacterial activity of G-11, its effect being greater than that of blood serum. When the susceptibilities of various strains were examined by the paper disc method, it was established that the Gram-positive cocci displayed a greater susceptibility to the action of G-11 than the Gram-negative rods. Decreasing the amount of antiseptic in the paper discs very slowly altered the antibacterial effect of G-11 as compared with phenol.

Soap containing 2 per cent G-11 has a marked degerming effect on the bacterial flora of the hands when it is used regularly and exclusively. A single brushless 3-minute scrub with soap containing 2 per cent G-11 does not seem to differ in efficiency from a routine surgical scrub with soap and brush followed by an alcohol rinse. This soap does not, however, appear to be significantly more effective than a soap containing 1 per cent G-11. It has been concluded that individual variations must be taken into account when assessing the degermation of the human skin by G-11.

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ERRATA

P. 5, line 7 from bottom: *for* 38 *read* 40
P. 10, Table 1: *for* 70 % ... 0.94 *read* 75 % ... 4.94
col. 2: *for* 9.3 *read* 5.3
P. 11, line 5 from bottom: *for* was *read* is
P. 15, lines 4 and 9: *for* Bonnet *read* Bonnett
line 18: *for* relapses *read* relapses of
P. 16, line 8: *for* stated *read* found
P. 17, line 2: *for* and brush *read* without a brush
line 2 from bottom: *delete* Murphy et al 1951,
P. 25, Table 3, line 2: *for* G-11 *read* potassium salt of G-11
P. 26, Fig. 3, line 2: *for* each of *read* of
line 3: *for* and Pseudomonas *read* and 10 strains of Pseudomonas
P. 34, line 6: *for* 0.05—0.02 *read* 0.03—0.02
P. 42, Table 13, line 1: *for* ih hand *read* in hand
P. 43, Table 14, col. 2: *for* 3.08 *read* 2.08
P. 45, Table 16, col. 6: *for* 5.13 *read* 6.13
col. 19: *for* 41 *read* 49
" *for* 63 ± 8.1 *read* 64 ± 7.4
P. 49, line 2 from bottom: *for* Bonnet *read* Bonnett